EXPERIMENTAL STUDY

Ultrastructural Changes of Schwann Cells during Nerve Regeneration Following a Crush Injury of the Sural Nerve in Rats

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Abstract

Twenty-four male albino rats (200 to 250g in weight) were used in the present study. The left sural nerve of 18 rats was subjected to crush injury while the sural nerves of 6 animals were used as control. After one week of the crush injury the Schwann cells showed multiple cytoplasmic processes. Those with long cytoplasmic processes wrapped only one axon while those wrapping multiple unmyelinated axons showed shorter processes. Some of these processes wrapped or surrounded collagen bundles "collagen pockets" and degenerated myelin, and some contained electronlucent vacuoles. Schwann cell cytoplasm showed asymmetric hypertrophy and contained dilated rough endoplasmic reticulum and ribosomes. Also, electronlucent vacuoles and whorls of degenerated myelin were seen in the cytoplasm of some Schwann cells. Schwann cells were surrounded by basal laminae which may be redundant. Two weeks post-crush, the number of regenerating Schwann cells increased and the myelin sheaths covering the myelinated axons were thicker. Schwann cells possessed long cytoplasmic processes that wrapped unmyelinated axons. After the third week of the crush injury, the Schwann cells wrapped shrunken myelinated axons with degeneration of the myelin of such axons. The number of myelinated axons increased, together with the thickness of their myelin sheath. It could be concluded that the Schwann cells play a phagocytic role during regeneration of peripheral nerves which is indicated by the presence of cytoplasmic vacuoles and degenerated myelin. Such phagocytic process might be performed by the use of their cytoplasmic processes.

Key words: Schwann cell, regeneration

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Introduction

It is well known that, unlike neurons in the central nervous system (CNS), neurons in the peripheral nervous system (PNS) have an intrinsic potential to regenerate upon crush injury or axotomy.

Upon peripheral nerve injury, a specific and orchestrated sequence of histopathological events takes place and eventually results in the full or partial regeneration of the injured nerve. In order to achieve a successful nerve repair, the injured tissue needs to be cleaned, and the axonal growth-inhibiting myelin debris must be removed [1]. Schwann cells are the main glial cells of the peripheral nervous system. They are responsible for the protection and support of the axons and for the synthesis of myelin. Consequently, the Schwann cells are important for nerve regeneration following nerve injury [2]. They play a crucial role in the process of axonal regeneration where nerve injury stimulates proliferation and activation of Schwann cells in the injured nerve fibers and synthesis of S-100 protein [3]. It has been shown that bone marrow stromal cells (BMSCs) can be induced to form Schwann cells by sequentially treating the cells with beta-mercaptoethanol and retinoic acid followed by forskolin and neurotropic factors including heregulin. The BMSCs-derived Schwann cells have a great potential to promote regeneration of peripheral nerves [4].

The intrinsic capacity of Schwann cells to promote regeneration after limited peripheral nerve lesions has been successfully transferred to extensive peripheral nerve injuries and central nervous system lesions by autologous transplantation of the Schwann cells aiming for axon repair and remyelination [5].

The aim of the current study was to examine the ultrastructural changes in Schwann cells during Wallerian degeneration after a nerve crush injury, in order to identify the main role of these cells in nerve degeneration and regeneration.

Materials and Methods

Animals and sural nerve crush

Twenty-four male albino rats aged thirty to forty days (200 to 250g in weight) were used in the present study. The left sural nerve of 18 rats is subjected to a crush injury applied by a non-toothed forceps for 10 seconds. The crush injury was performed, at week-intervals, on 6 animals each week. The right and left sural nerves of the remaining 6 rats were used as control. The rats were initially anaesthetized by ether inhalation as induction anaesthesia, then by an intraperitoneal injection of pentabarbitane sodium (Sagatal; 60 mg/ml) at a dose of 30 mg/kg body weight. Anaesthesia was maintained during surgery by repeated intraperitoneal injection of pentabarbitane sodium. The left sural nerve was exposed through an incision overlying the left popliteal fossa and runs parallel to the short saphenous vein.

Collection of specimens and tissue processing

At set times after nerve crush ranging from one to 3 weeks, the animals were reanaesthestized initially by ether inhalation then weighed and injected with pentabarbitane sodium according to their weight. The crushed nerves were exposed and fixed locally using 4%

paraformaldehyde and 2.5 % glutaraldehyde. The specimens of the crushed nerves distal to the site of the crush and normal nerves were removed and put in the fixative for 3 hours at 4° C. The nerves were segmented into 2.5 mm segments. Each segment was put in a separate fixative-filled vial and labeled. The specimens were then put in the fixative for up to four hours then transferred to phosphate or cacodylate buffer solution and left over night. They were dehydrated in ascending grades of alcohol then put in two changes of propylene oxide for 5 minutes each.

Results

I. The control group

Control sections showed Schwann cells wrapping myelinated and unmyelinated axons. The Schwann cells were surrounded by distinct basal laminae that showed no redundancy. Their surfaces were regular without marked cytoplasmic processes. Their cytoplasm showed normal content of cell organelles including mitochondria and ribosomes. Moreover, the Schwann cells were surrounded by compact bundles of collagen fibrils (**Figure 1**).

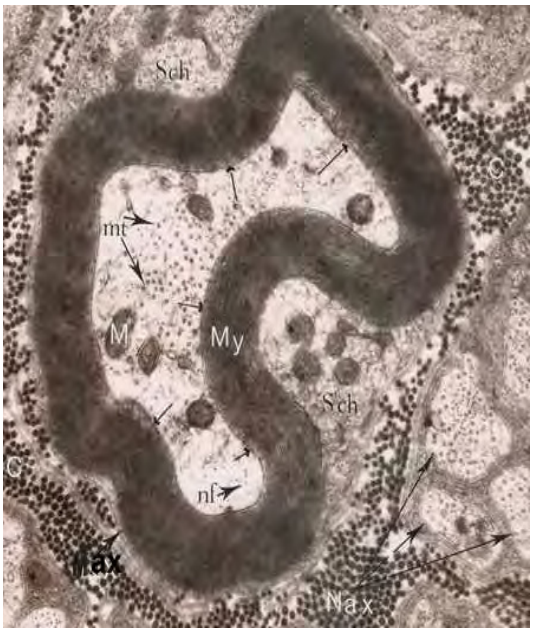

Figure 1. *An electron photomicrograph of a transverse section of a normal sural nerve. It shows a myelinated axon (Max) wrapped by a Schwann cell (Sch). The Schwann cell shows distinct basal lamina and normal cytoplasmic organelles such as mitochondria. The figure also shows a Schwann cell wrapping many unmyelinated axons (Nax). Compact collagen bundles (C)fill the endoneurium. My (myelin sheath), mt (microtubules), M (mitochondria), nf (neurofilaments) X 40000*

II. The crushed nerves

A. One week post-crush

Some of the examined sections showed areas of regenerating axons. The Schwann cells exhibited multiple cytoplasmic processes which varied in length. Those with long cytoplasmic processes wrapped only one axon, while those cells which wrapped multiple unmyelinated axons showed shorter processes. Some of these processes wrapped or surround collagen bundles "collagen pockets" and degenerated myelin. Other processes contained electrolucent vacuoles. The Schwann cell cytoplasm showed asymmetric hypertrophy and contained dilated rough endoplasmic reticulum and ribosomes. Also, electronlucent vacuoles and whorls of degenerated myelin were seen in the cytoplasm of some Schwann cells.

The Schwann cells were surrounded by basal laminae that covered the external surface and did not extend to surround the invaginated processes or the axons (**Figure 2**).

The basal laminae covering the basal Schwann cells that wrapped the regenerating myelinated and unmyelinated axons appeared redundant and excess parts extended into the endoneurium (**Figure 3**).

Some of the recovering Schwann cells contained a large number of the

electronlucent fat vacuoles, which were not membrane bounded, and electron dense whorls of degenerated myelin. Their cytoplasm contained regenerating unmyelinated axons. The features are shown in **Figure 4.**

B. Two weeks post-crush

The number of the regenerating Schwann cells increased and the myelin sheaths covering the myelinated axons appeared thicker.

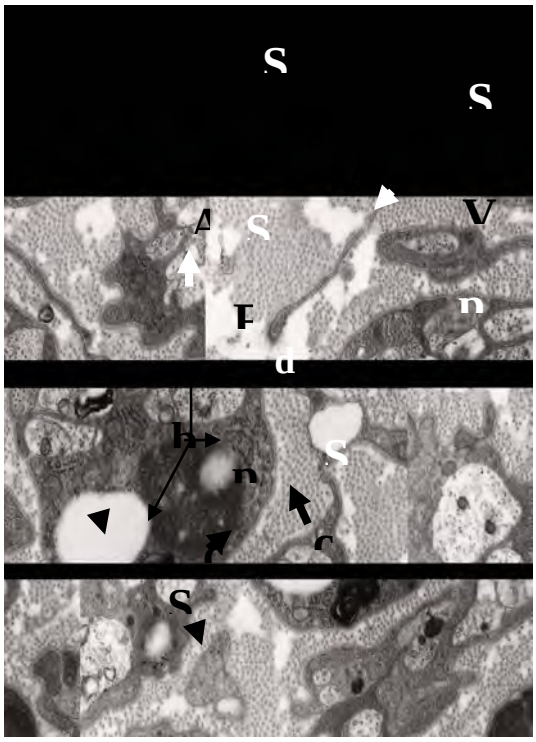

Figure 2. *An electron photomicrograph of a transverse section of a sural nerve one week after crush injury. It shows a Schwann cell (Shw) exhibiting a long cytoplasmic process (p) which contains electronlucent fat vacuole (V). Another Schwann cell (Sh) shows multiple cytoplasmic processes (p) that encircles part of the endoneurium containing collagen fibrils (c). A third Schwann cell (Sch) wraps many unmyelinated axons (A), its cytoplasm contains a large electronlucent fat vacuole (F), whorls of degenerated myelin (dm) and dilated rough endoplasmic reticulum (arrows). A fourth Schwann cell (SW) shows asymmetric hypertrophy of cytoplasm which contains excess amount of ribosomes that form polysomes (arrowheads). All Schwann cells are covered externally with basal lamina (bl) X 32000.*

The Schwann cells possessed long cytoplasmic processes that wrapped unmyelinated axons.

Some of them contained cytoplasmic electronlucent fat vacuoles and whorls of electrondense degenerated myelin (**Figure 5**).

C. Three weeks post-crush

Some sections showed Schwann cells wrapping shrunken myelinated axons with degeneration of the myelin of such axons.

Figure 3. *An electron photomicrograph of a transverse section of a sural nerve one week after crush injury. It shows a Schwann cell (Sch) wrapping a single thinly myelinated axon (AX). Other Schwann cells wrap the multiple unmyelinated axons (N). The basal laminae covering the Schwann cells appear redundant (arrow heads). m (mitochondria), c (collagen fibrils), nf (neurofilaments), arrows (microtubules) X 32000*

The cytoplasm of these Schwann cells contained excess amounts of vacuoles of different sizes and shapes, most of them were membrane-bounded, probably degenerating vacuoles. The cytoplasm did not show many cell organelles. The basal lamina of some of these Schwann cells were well-defined

and intact. The described changes are demonstrated in (**Figure 6**).

The number of myelinated axons increased and the thickness of the myelin sheath also increased. The Schwann cells showed asymmetrical hypertrophy of their cytoplasm with increased number of mitochondria and dilated rough endoplasmic reticulum, which showed wide cisterns (**Figure 7**).

Figure 4. *An electron photomicrograph of a transverse section of a sural nerve one week after the crush injury. It shows a Schwann cell (S) containing whorls of degenerated myelin (m & D) and fat vacuoles (f). The Schwann cell wraps a regenerating unmyelinated axon (R). Other Schwann cells wrap many unmyelinated axons (n) and possess many cytoplasmic processes. F (fibroblasts), E (rough endoplasmic reticulum), T (contact between fibroblasts) X 10000*

Discussion

The current study showed that after one week of the crush injury the Schwann cells exhibited a number of morphological changes. They showed extended multiple short and long cytoplasmic processes. The long cytoplasmic processes contained electronlucent fat vacuoles, and some of them encircled collagen fibrils forming collagen pockets and also encircled parts of the endoneurium.

Some Schwann cells contained large amount of electronlucent fat vacuoles and degenerated myelin. Consequently, it appears that Schwann cells play a strong phagocytic role during Wallerian degeneration, in order to remove myelin

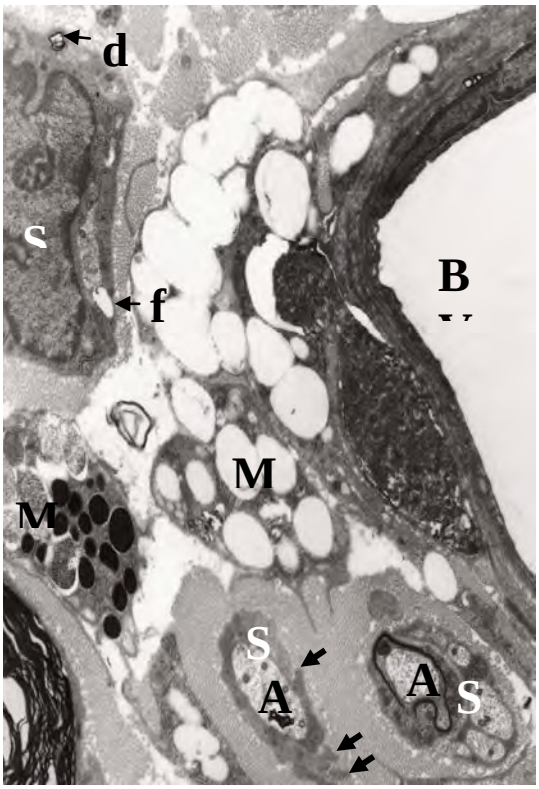

Figure 5. *An electron photomicrograph of a transverse section of a sural nerve two weeks after the crush injury. It shows a Schwann cell (Sc) with multiple cytoplasmic processes (arrows) and wraps a single unmyelinated axon (A). Another Schwann cell (S) wraps thinly myelinated axon (AX). A third Schwann cell (SH) contains an electronlucent fat globule (f) and degenerated myelin (d). BV (blood vessel) MG (endoneurial macrophage) M (mast cell) X 25000*

Figure 6. *An electron photomicrograph of a transverse section of a sural nerve three weeks after the crush injury. It shows a Schwann (Sch) wrapping a shrunken myelinated axons (Ax) with degeneration of its myelin (DM). The Schwann cell cytoplasm contains membrane-bound vacuoles (arrows). The Schwann cell basal lamina (bl) is well-defined. X 20000*

Figure 7. *An electron photomicrograph of a transverse section of a sural nerve three weeks following the crush injury. It shows a Schwann cell (Sch) which contains a large number of mitochondria (m) and dilated rough endoplasmic reticulum (arrows). F (fibroblast) Ax (myelinated axon) X 40000.*

debris and fat globules. They probably use their cytoplasmic processes to phagocytose the degenerated myelin. It has been reported that Schwann cells become dedifferentiated or activated in response to a nerve injury [6]. The activated Schwann cells then retain their phagocytic capacity and begin cleaning the myelin and dead neuronal debris [7].

In this cleaning process, the Schwann cells express various cytokines and chemokines, such as TNF-a IL-6, and MCP-1, which recruit macrophages from the blood vessels and induce local inflammation [8, 9].

In addition, peripheral nerve injury induces iNOS gene expression in Schwann cells. The production of nitric oxide in the PNS is implicated in the nerve injury-mediated neuropathic pain[10]. However, it is unclear how Schwann cells recognize the nerve damage and become activated. Recently, it was reported that necrotic cell-derived endogenous molecules, such as heat shock proteins (Hsp) and RNA, activate nearby innate immune cells by binding to toll-like receptors (TLRs) [11, 12]. TLRs are type I trans-membrane proteins that are known to recognize the specific molecular structures originating from microorganisms, thereby inducing inflammatory responses.

Thus far, more than 10 different TLRs with distinct legend specificity have been identified [13, 14]. In the PNS, it was recently reported that TLR2 and TLR3 are expressed in the Schwann cells [15, 16]. Other researchers [17] postulated that damaged neuron-derived molecules induce inflammatory Schwann cell activation during Wallerian

degeneration and tested this hypothesis by using necrotic neuronal cells (NNC). The results show that NNC stimulation induces TNF-a and iNOS gene expression in Schwann cells via TLR2 and TLR3. This suggests that endogenous TLR2 and TLR3 agonists, released from an injured nerve, may activate Schwann cells to express pro-inflammatory genes during Wallerian degeneration.

The current study also showed that some Schwann cells wrapped only one axon, while others wrapped many unmyelinated axons. Also it was found that Schwann cells wrapped only regenerated myelinated axon. It is unclear which of the regenerating axons was chosen by Schwann cells to be singly wrapped and then myelinated. It was reported that following injury, axons distal to the injured site degenerate and the myelin sheaths break down. Schwann cells dedifferentiate and proliferate then they stop proliferation and start remyelination [18].

It was also reported that Schwann cells during the embryonic development differentiate into non-myelinating and myelin-forming Schwann cells in the postnatal period [19]. It could be inferred that the same occurs during nerve regeneration and that Schwann cells will differentiate into myelin - forming and non-myelinating Schwann cells. The myelin-forming cells will wrap only one axon while the non-myelinating cells will wrap several regenerating axons.

The molecular mechanisms that regulate these processes are partially understood. The myelination program is crucially dependent on the expression of at least two transcription factors oct6/Scip/Tst 1 [20] and Krox 20/Egr2 [21]. Myelination is delayed with deficiency of oct6/Scip/Tst 1 [22], while compact myelin will never be formed with deficiency of Krox 20/Egr2.[23] Regulated exit from cell cycle is a prerequisite for the Schwann cells to achieve myelination. Understanding the underlying signals may have practical implications for treatment of peripheral nerve tumors (Schwann cell hyperplasia), peripheral neuropathies secondary to diabetes, cancer chemotherapeutic agents, toxins, and autoimmune disorders [24]. Aberrant Schwann cell proliferation is also a prominent feature in inherited peripheral neuropathies [25, 26]. The current study showed also that Schwann cells exhibited redundant basal laminae. Excess basal laminae in the current study extended into the endoneurium in the form of electrondense membranes. It could be suggested that after the developing axons reach their final size, the excess basal laminae will be detached into the endoneurium either to be recycled or to degenerate.

Regenerating Schwann cells in the current study showed asymmetric hypertrophy of their cytoplasm. The cytoplasm contained dilated rough endoplasmic reticulum which was not encountered frequently in the normal control Schwann cells. This is probably for manufacture of more proteins to rebuild the regenerating axons or for the formation of factors necessary for the growth of the wrapped axons. Their cytoplasm also contained excess free ribosomes and polysomes for manufacture of proteins necessary for the growth of the regenerating Schwann cells and for myelination of the wrapped axons.

It could be concluded that Schwann cells exhibit many ultrastructural changes during regeneration of the peripheral nerves, including the

formation of long and short cytoplasmic processes and redundant basal laminae. A phagocytic role of Schwann cells is suggested in view of the presence of cytoplasmic vacuoles and whorls of degenerated myelin. Such phagocytic process might be performed by the use of the cytoplasmic processes.

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